CLOSTRIDIUM KLUYVERII, AN ORGANISM CONCERNED IN THE FORMATION OF CAPROIC ACID FROM ETHYL ALCOHOL

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When ethyl alcohol is acted upon by the methane-producing organism, *Methanobacterium omelianskii*, acetic acid is the sole oxidation product (Barker (1939–40, 1941)). But with alcohol-containing enrichment cultures for the same organism it often happens that acetic acid is formed in only relatively small amounts, while most of the alcohol is converted into caproic and butyric acids (Barker (1937)). In some cultures as much as 75 per cent by weight of the volatile acid is caproic.

Microscopic examination has shown that such caproic-acid-producing enrichment cultures always differ from those forming only acetic acid by containing, besides *M. omelianskii*, considerable numbers of a large, motile, spore-forming bacterium. The conclusion seemed justified that this latter organism either itself produces or at least helps to produce the caproic acid. However, direct proof of the participation of the spore-forming bacterium in caproic acid formation can be obtained only by studying the organism in pure culture.

This communication describes the isolation, the general morphological and physiological characteristics and certain growth requirements of this bacterium. Also, evidence is presented which demonstrates that it is responsible for the conversion of ethyl alcohol to caproic acid.

Enrichment and isolation. To obtain the caproic acid-producing organism, *Clostridium kluyverii*,[1] the following enrichment medium (no. 1) made with tap water is used: C_2H_5OH 1 vol. per cent; K_2HPO_4 0.5 per cent; $MgSO_4 \cdot 7H_2O$ 0.01 per cent; $(NH_4)_2SO_4$ 0.03 per cent; $FeSO_4 \cdot 7H_2O$ 0.002 per cent; yeast autolysate 0.5

[1] Justification for the use of this name is given in a later section of this paper.

vol. per cent, and $CaCO_3$ about 10 per cent. The yeast autolysate, though not essential, sometimes appears to be beneficial. After autoclaving the medium, 2 vol. per cent of a 5 per cent Na_2CO_3 solution and 1 vol. per cent of a 1 per cent $Na_2S \cdot 9H_2O$ solution are added. The reaction is then adjusted to pH 7.0–7.4. A heavy (5 per cent) inoculum of black mud from fresh water or marine sources is added and the cultures are incubated at 35°C. in completely filled glass-stoppered bottles in order to exclude oxygen.

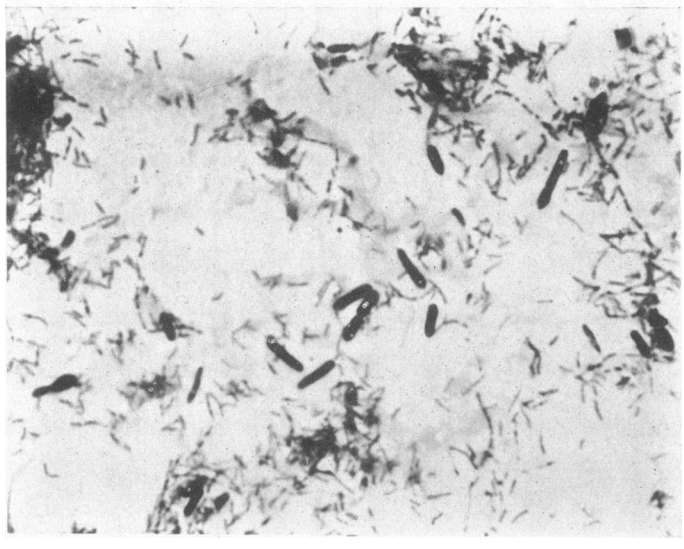

FIG. 1. CAPROIC-ACID-PRODUCING ENRICHMENT CULTURE CONTAINING M. OMELIANSKII (SMALL RODS) AND C. KLUYVERII (LARGE SPORE-FORMING RODS)

In such enrichment cultures *M. omelianskii* always develops abundantly within a few days with the formation of acetic acid and methane. After about a week the cultures also frequently develop a characteristic odor of higher fatty acids and alcohols which indicate the presence of *C. kluyverii* (fig. 1). The fatty acids of higher molecular weight than acetic can be easily demonstrated by the Duclaux distillation method (Knetemann) after they have been separated from the medium by steam distillation. Distillation data for several enrichment cultures and for pure fatty acids are given in table 2.

Before undertaking the isolation of *C. kluyverii* it is desirable to transfer the enrichment cultures one or two times. Like original cultures, the transfers form caproic acid only slowly; each should be incubated for at least 1 to 2 weeks.

A solid medium of the same composition as the enrichment medium proved to be unsatisfactory for the isolation of *C. kluyverii* by the shake-culture technique since colonies of this organism were never obtained beyond the fourth dilution although *M. omelianskii* developed at much higher dilutions. Furthermore, transfers to a second series of shake cultures of the same medium invariably failed to produce growth. These observations, coupled with the already mentioned fact that *C. kluyverii* does not appear in enrichment cultures until after an abundant growth of *M. omelianskii*, suggested that the development of the former organism in the relatively simple enrichment medium is in some way dependent upon the presence of the latter organism. This conclusion was amply verified by subsequent pure culture studies.

After numerous unsuccessful attempts it was found that a satisfactory development of *C. kluyverii* could be obtained in a medium (no. 2) containing 20 vol. per cent yeast autolysate, 0.5 vol. per cent C_2H_5OH and the inorganic constituents of medium 1. In making isolations an enrichment culture containing mature spores of *C. kluyverii* is chosen, a sample of the sediment is heated for 15 minutes at 80°C. and inoculated into a series of shake cultures. The tubes are closed with a pyrogallol-K_2CO_3 seal and incubated at 35°C.

Since the isolation medium is not selective, considerable care must be taken in the examination of developing colonies if the isolation is to be successful. The lower dilutions are invariably contaminated with fast-growing anaerobes, some of which produce sufficient gas to split the agar within 24 hours. Such tubes may be discarded immediately since *C. kluyverii*, though present, is completely overgrown. In the higher dilutions fast-growing organisms may also develop but they are not a source of serious difficulty as long as they remain isolated in the agar. Here, *C. kluyverii* is frequently the most abundant organism. Generally it may be recognized by its slow growth and small gas production.

Small colonies begin to appear only after the second or third day of incubation; gas bubbles seldom appear until the 4th or 5th day, if at all. The colonies are generally small (1–3 mm.), fluffy spheres composed of filamentous outgrowths from a rather dense central nucleus. However, compact lens-shaped colonies may also occur (fig. 2). The consistency of the colonies is such that they may be easily removed with a micropipette.

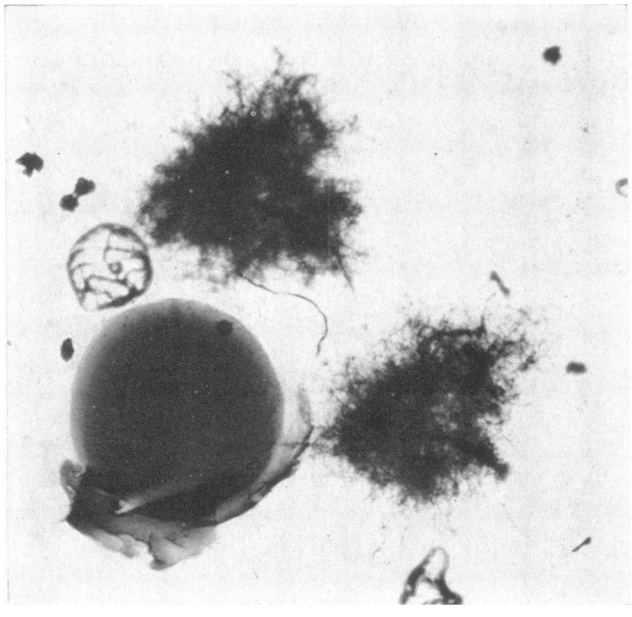

FIG. 2. TWO TYPES OF DEEP AGAR COLONIES FORMED BY C. KLUYVERII, STRAIN K21
Medium 2. Two weeks old. × 20

When isolated colonies of the desired organism have been located, pure cultures may be obtained without difficulty by using the shake culture technique and the medium described above. Nine strains have been isolated by us. They are kept in stab cultures protected by pyrogallol seals.

Morphology and growth characteristics. All nine strains are morphologically very similar to strain Kl, pictured in figure 3. The cells are about 0.9–1.1 microns by 3–11 microns. They occur

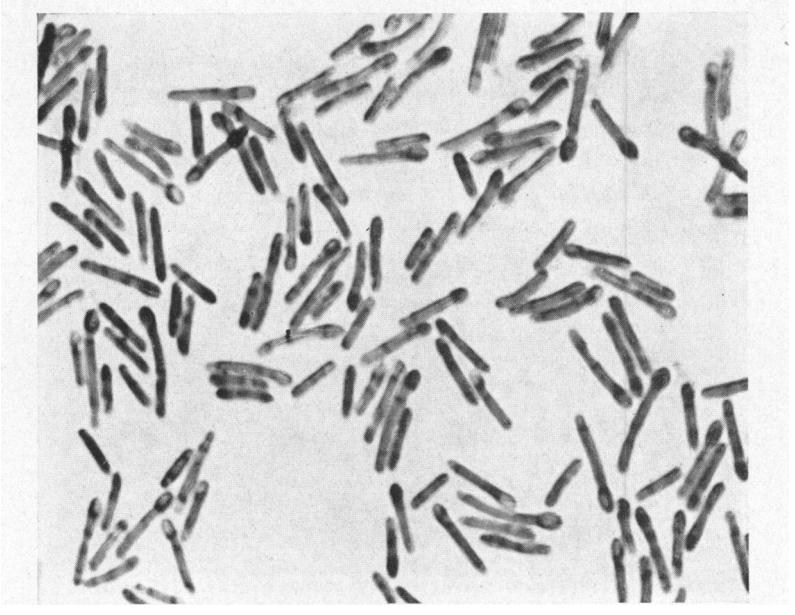

FIG. 3. VEGETATIVE AND SPORULATING CELLS OF C. KLUYVERII, STRAIN K1
Erythrosine stain. × 1600

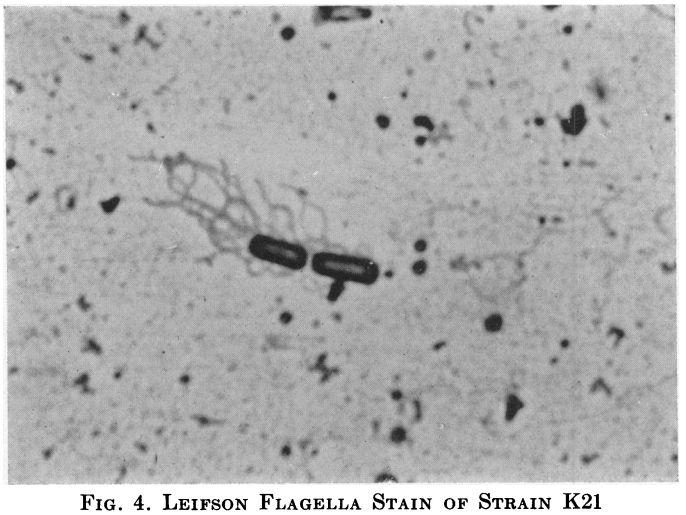

FIG. 4. LEIFSON FLAGELLA STAIN OF STRAIN K21
× 1900

singly, in pairs, or sometimes in fairly long chains. Spores are usually terminal, oval in shape (1.3 microns by 1.8 microns) and cause a slight swelling of the rod. When young, the cells are motile by means of peritrichous flagella (fig. 4). They stain readily by most methods, but are generally gram-negative both in young and old cultures.

Freshly isolated organisms form mostly rough colonies; however, after prolonged cultivation, some strains at least (K1 and K2) also form smooth, compact colonies. Cells from the two

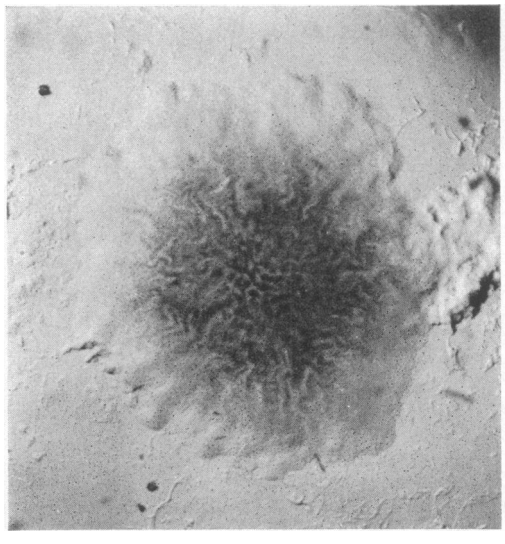

FIG. 5. SURFACE COLONY OF STRAIN K1
Medium 2. × 20

colony types are microscopically indistinguishable. The rough type of strain K21 produces a diffuse turbidity in liquid media during the active phase of growth, whereas the smooth type grows as a sediment, leaving the liquid almost perfectly clear. Surface colonies of either type can be obtained only under strict anaerobiosis (fig. 5).

As has been previously mentioned, *C. kluyverii* grows slowly. In liquid, semisolid or solid media visible growth never appears in less than 48 to 72 hours incubation at 37°C. Surface growth on

solid media is frequently delayed much longer, possibly due to the inhibiting action of traces of oxygen.

PHYSIOLOGY

Growth conditions. The organism is an obligate anaerobe. No growth occurs on aerobic plates or in the oxidized zone of methylene-blue-containing stab cultures exposed to air. It is desirable therefore to add a reducing agent such as hydrogen sulfide or thioglycollate (0.01–0.05 per cent) to all media, although such additions are not strictly necessary when high concentrations of yeast autolysate (10–30 vol. per cent) are used.

The pH range for growth is from 6.0 to 7.5, the optimum being at about pH 6.8. To maintain the desired pH, potassium phosphate buffers may be used at concentrations as high as 1.5 per cent without deleterious effects. Growth occurs from 19–37°C.; lower temperatures were not tested. Growth is most rapid at about 34°C. All these relations were established by the use of medium 2 and strain K1.

The influence of the concentration of yeast autolysate on growth is of special interest. Although all strains were originally obtained from enrichment media containing little or no yeast autolysate, it has been repeatedly observed that in pure culture they do not develop in medium 2 when the yeast autolysate is omitted. Small additions of yeast autolysate (1–3 vol. per cent) do not exert any observable effect. Only at a minimum concentration of about 5 vol. per cent, the exact concentration varying with the batch of yeast autolysate, does visible growth occur; it is greatly increased by further additions up to at least 20–30 vol. per cent. Table 1 illustrates these relations and also shows that neither tryptone (Difco) nor proteose peptone (Difco) can replace yeast autolysate.

Although a medium containing 20–30 vol. per cent yeast autolysate is satisfactory for the isolation and propagation of *C. kluyverii*, the yield of cells is much smaller than with other anaerobic bacteria growing under comparable conditions. This indicates that even the yeast autolysate medium is not optimal. An effort was therefore made to find a better medium.

The following media were tried: (a) 1 per cent Difco peptone, (b) 1 per cent Difco tryptone plus 1 per cent glucose, (c) 1 per cent tryptone, (d) 1 per cent Difco proteose peptone, (e) 5 vol. per cent yeast autolysate, (f) 5 vol. per cent yeast autolysate plus 0.5 per cent glucose, (g) Difco brain-liver-heart medium, (h) 1 per cent Difco liver-veal extract, (i) 5 per cent Difco malt extract, (j) neutralized tomato juice, (k) iron-milk (Spray (1)), (l) iron-gelatin (Spray (1)), (m) brain broth. *C. kluyverii* did not grow in a, b, c, d, h, i, j, k, or l. Though some growth did occur in e, f, g and m, it was never as abundant as in medium 2. The results show that *C. kluyverii* is unable to use either certain carbohydrates or

TABLE 1

Effects of yeast autolysate, tryptone, and peptone upon growth

Modified medium 2, semi-solid. Incubated for 9 days at 37 C. in tubes exposed to the air

COMPLEX NUTRIENT	RELATIVE GROWTH		
	K1	K6	K13
5 vol. per cent yeast autolysate	−	−	−
10 vol. per cent yeast autolysate	−	+	+
20 vol. per cent yeast autolysate	+++	+++	+++
30 vol. per cent yeast autolysate	+++	+++	+++
5 vol. per cent yeast autolysate + 1 per cent tryptone	−	−	−
5 vol. per cent yeast autolysate + 1 per cent proteose peptone	−	−	−

products of protein hydrolysis under conditions suitable for the growth of most species of *Clostridium*. Yeast autolysate evidently has special value as a nutrient for *C. kluyverii*; it must provide some essential substance or group of substances of undetermined nature not present in the other substrates tested.

Metabolic activities. Because of the complexity of the media in which *C. kluyverii* grows in pure culture it has not yet been possible to determine the chemical reactions by which it obtains energy. Progress has been made, however, by identifying at least some of the compounds produced or consumed during growth in medium 2. This was done by analyzing cultures before and after incubation.

Ammonia. Since *C. kluyverii* requires yeast autolysate, a material rich in nitrogenous compounds, one might expect ammonia to be an important metabolic product. Actually, little or no ammonia is liberated during growth; a small uptake of ammonia was even observed in one experiment. These results definitely indicate organic nitrogenous compounds are not the principal substrates of *C. kluyverii.*

Gases. In medium 2 the organism always produces a little gas consisting of hydrogen and carbon dioxide (about 15 per cent). The latter is derived mostly from the added bicarbonate; a very

TABLE 2

Duclaux distillation data for pure fatty acids and for volatile acids from cultures of C. kluyverii

Total vol. = 110 cc. The results are expressed as percentages of the acid in the first 100 cc. of distillate

SOURCE OF VOLATILE ACID	MILLILITERS OF DISTILLATE			
	20	40	60	80
Acetic (C.P.)	15.5	32.5	51.3	72.3
Valeric (E. K. Co.)	43.5	70.9	89.3	95.6
Caproic (E. K. Co.)	56.7	83.7	94.8	98.8
Enrichment culture 1	23.3	43.2	61.8	80.0
Enrichment culture 2	40.0	64.0	81.5	92.0
Enrichment culture 3	44.1	68.1	83.7	92.8
Pure culture strain K1	44.8	71.8	83.6	91.8
Pure culture strain K21	53.3	73.2	85.2	94.4

little carbon dioxide may also be formed during the fermentation. Methane is not produced.

Fatty acids. C. kluyverii does not produce the offensive putrefactive odors characteristic of many species of *Clostridium.* A rather strong odor of caproic and butyric acids and the corresponding alcohols is present. This odor is so distinctive that it proved very useful in identifying the organism during the early attempts to isolate pure cultures.

The similar odors of the pure and enrichment cultures suggested that the former as well as the latter are able to form higher fatty acids. This suggestion was verified by examining the volatile

acids by the Duclaux distillation method. Although the volatile acids were always produced in relatively small amounts (1.5–12.3 mM/L), the Duclaux data leave no doubt that they consist largely of an acid more volatile than valeric acid (table 2). This is probably caproic acid in view of the results previously reported with enrichment cultures (Barker (1937)). The unfermented media contained acetic acid as the only volatile acid.

Ethyl alcohol. Since enrichment cultures of *C. kluyverii* convert ethyl alcohol into caproic acid, it might be expected that alcohol would also be used by pure cultures. Experiments with two strains (K1 and K21) have shown that alcohol does disappear during growth, though here again the amount is small (10–22 mM/L). Alcohol was determined by the method of Northrop.

Non-volatile acids. Yeast autolysate always contains a considerable amount of non-volatile, ether-soluble acids. These decrease during fermentation. Because the most abundant non-volatile acid in yeast autolysate is lactic acid, it seems probable that this is the compound utilized, although direct evidence on this point has not been obtained.

Quantitative fermentation experiments. Although ethyl alcohol and non-volatile acids disappear and caproic acid is produced during fermentation, it does not necessarily follow that the former compounds are converted into the latter. In the complex medium used, the caproic acid might also originate from the yeast autolysate. The presumption that caproic acid is derived from ethyl alcohol (and possibly also from lactic acid) was originally based on experiments with enrichment cultures containing no yeast autolysate. This view has been materially strengthened by the observation that the quantity of caproic acid produced is approximately equivalent to the alcohol and lactate consumed.

The results of a quantitative fermentation experiment with the smooth variant of strain K21 are given in table 3. Ethyl alcohol was determined by Northrop's method on the assumption that no other oxidizable, neutral-volatile substance was present. Actually the fermented medium also contained a small amount of higher alcohols. The total non-volatile acid was assumed to be

lactic acid. Acetic and caproic acids were determined by the Duclaux distillation method on the assumption that no other volatile acids were present. Since some butyric acid may have been formed, this method of calculation tends to make the results for acetic and caproic acids somewhat too high.

Despite the above mentioned analytical uncertainties and the complexity of the medium, the conclusion seems justified that ethyl alcohol and caproic acid are the main substrate and product, respectively. It is evident, however, that part of the caproic acid

TABLE 3

Chemical changes resulting from the growth of C. kluyverii in medium 2

Strain K21, smooth variant. Incubated at 30°C. for 8 days

SUBSTANCE	mM/L	PER CENT CARBON	PER CENT AVAILABLE HYDROGEN
Substrates:			
Ethyl alcohol	−22.0	66.6	74.8
Lactic acid	−5.5	25.3	19.1
Acetic acid	−2.7	8.1	6.1
Total		100	100
Products:			
Ammonia	0.21		
Hydrogen	0.35		0.2
Caproic acid	11.6	106	106.1
Total		106	106.3

also comes from the non-volatile acid. Although acetic acid also appears to be decomposed, the possible experimental error in the determination of this compound is so considerable that a final decision as to its utilization must await further work. Hydrogen and ammonia are quantitatively very minor products. Carbon dioxide was not formed in significant amounts in this fermentation.

Two other quantitative fermentation experiments with strain Kl and the rough variant of strain K21 gave essentially similar results.

CHEMICAL ACTIVITIES OF C. KLUYVERII GROWING IN ASSOCIATION WITH M. OMELIANSKII

Because caproic acid is produced in considerably larger quantities by enrichment cultures containing both *C. kluyverii* and *M. omelianskii* than by pure cultures of the former organism it seemed of interest to study volatile acid formation when pure cultures of both organisms are simultaneously inoculated into alcohol-containing media. The two organisms acting together might produce caproic acid when neither organism alone could do so.

The organisms were inoculated into a mineral-alcohol medium (no. 3) of the following composition: agar 0.2 per cent; C_2H_5OH 1 vol. per cent; K_2HPO_4 0.6 per cent; KH_2PO_4 0.4 per cent; $(NH_4)_2SO_4$ 0.03 per cent; $MgSO_4 \cdot 7H_2O$ 0.01 per cent; $FeSO_4 \cdot 7H_2O$ 0.001 per cent, and $CaSO_4$ 1 vol. per cent of a saturated solution. After autoclaving, 0.01 per cent $Na_2S \cdot 9H_2O$ and 0.2 per cent Na_2CO_3 were added as sterile solutions and the reaction was adjusted to pH 7.2 with sterile HCl solution. The medium was incubated at 37°C. in test tubes protected from oxygen by a pyrogallol-K_2CO_3 seal. This medium is admirably suited to *M. omelianskii* but does not allow the development of *C. kluyverii* in pure culture. When inoculated with both organisms growth became visible after two days and, subsequently, considerable gas and volatile acid were produced. Microscopic examination showed, however, that only *M. omelianskii* grew well. The chemical changes also were characteristic of the methane-producing bacterium; the volatile acid was all acetic acid.

A second similar experiment was carried out in which medium 3 was supplemented by the addition 5 vol. per cent yeast autolysate. It will be recalled that this quantity of yeast autolysate allows only a very limited growth of *C. kluyverii* in pure culture. In the present experiment the cultures inoculated with both organisms developed well and after a week possessed the odor characteristic of *C. kluyverii*. Microscopic examination showed large numbers of both organisms, and the volatile acids contained about 75 per cent by weight of caproic acid. It is evident, therefore, that *C. kluyverii* develops much better in a medium with 5 per cent yeast autolysate plus *M. omelianskii* than with yeast autolysate alone.

In order to examine further the influence of yeast autolysate on the growth of *C. kluyverii* alone and with *M. omelianskii*, another experiment was set up in which the yeast autolysate content of medium 3 was adjusted at 0, 1, 3, 9, and 27 vol. per cent. One series of cultures was inoculated with *C. kluyverii* alone, a second series with both organisms. After 2 to 3 days incubation at 37°C. growth was visible in all tubes containing *M. omelianskii*, while with *C. kluyverii* alone growth occurred only with 9 and 27 vol. per cent yeast autolysate. These results confirmed the previous experiments. After 7 days incubation volatile acids were deter-

TABLE 4

Influence of yeast autolysate on caproic acid formation by C. kluyverii (strain K1) growing alone and in association with Mb. omelianskii

(Quantities of acids are expressed in mM/liter)

ORGANISMS	YEAST AUTOLYSATE	GAS PRODUCTION	FINAL pH	TOTAL VOLATILE ACID	C_2H_5OH EQUIVALENT OF VOLATILE ACIDS		
					Acetic*	Caproic	Total
	vol. per cent						
Cl. kl. + Mb. om.	0	+++	5.8	39.3	39.3	0.0	39.3
	1	+++	6.0	40.4	14.0	79.0	93.1
	3	+++	6.0	41.3	14.3	81.0	95.3
	9	+++	5.8	46.6	19.6	73.6	93.2
	27	+	7.0	18.6	5.2	40.2	45.4
Cl. kl. alone	9	±	7.2	5.4	+	+	
	27	+	7.0	15.7	2.6	39.3	41.9

* No correction has been made for acetic acid in the added yeast autolysate.

mined with the results recorded in table 4. The relative amounts of gas produced and the acidities of the fermented media are also indicated.

In discussing the data of table 4 it will be convenient to deal first with the cultures containing both organisms and 0–9 vol. per cent yeast autolysate. These cultures differed from the others in producing more gas and more volatile acid and in having a lower final pH. The gas undoubtedly consisted mainly of methane, since microscopic examination showed the presence of large numbers of *M. omelianskii*. While the volatile acid increased somewhat

with the yeast autolysate concentration, the final acidities of these four cultures did not differ appreciably, probably because the buffer capacity was raised simultaneously. It should be noted, however, that acidity was undoubtedly the limiting factor in these fermentations. In the absence of yeast autolysate *C. kluyverii* failed to develop and acetic acid was the only volatile acid formed. But with the addition of only 1 per cent yeast autolysate, an amount entirely inadequate to permit the growth of this organism in pure culture, a relatively large amount of caproic acid was produced, while at the same time the yield of acetic acid was decreased by about 65 per cent. The quantity of ethyl alcohol converted was more than twice as great in the presence as in the absence of yeast autolysate (last column, table 4). Further increases in yeast autolysate concentration up to 9 vol. per cent did not greatly alter the yields of either acid or improve the growth of *C. kluyverii*.

The cultures containing both organisms and 27 vol. per cent yeast autolysate behaved quite differently from those with lower concentrations. Much less gas was produced, the pH dropped very little and the yields of caproic acid and, more particularly, of acetic acid were much lower; all these things indicate that the chemical changes were due largely to *C. kluyverii*. This was confirmed by microscopic examination and by comparison of the volatile acids, pH and gas production with those obtained in the same medium inoculated with *C. kluyverii* alone. Evidently the high concentrations of yeast autolysate, most favorable for *C. kluyverii*, are toxic to *M. omelianskii*.

Several significant conclusions may be drawn from this experiment. Firstly, the results confirm the view that *C. kluyverii* is responsible for the formation of caproic acid and that this compound is derived from ethyl alcohol. The previous evidence on these points was incomplete, since, though in the experiments with enrichment cultures (Barker (1937)) it was clear that ethyl alcohol was converted into caproic acid, the causative organism was not positively identified; and in the experiments with pure cultures of *C. kluyverii*, using media containing high concentrations of yeast autolysate, though caproic acid was definitely formed, it

could not be conclusively proved to originate from alcohol. Now both points have been verified simultaneously.

Secondly, it is evident that caproic acid formation by *C. kluyverii* is greatly favored by the presence of *M. omelianskii*. While the mechanism of this beneficial effect is not clear, it appears to depend upon a chemical synergism of the two organisms. The formation of caproic acid from alcohol involves a net oxidation and the only oxidizing agent available in appreciable quantities in the medium containing 1 vol. per cent of yeast autolysate is carbon dioxide. Furthermore, it has been shown (Barker (1937)) that more methane is formed in caproic-acid-producing cultures than is required for the oxidation of alcohol to acetic acid. It is reasonable to suppose, therefore, that the function of *M. omelianskii* is to carry out some essential oxidation reaction with carbon dioxide as the hydrogen acceptor. Whether this oxidation reaction is the conversion of alcohol to acetaldehyde as previously suggested still remains to be determined. A logical consequence of this view of the function of the methane-producing bacterium is that in its absence some oxidizing agent other than carbon dioxide must be supplied. Possibly the yeast autolysate contains small quantities of some compound which fulfills this function. The high requirement of pure cultures for this material might thus be explained.

Thirdly, one may conclude that *C. kluyverii*, even when growing in the presence of *M. omelianskii*, does require a small amount of yeast autolysate. This was somewhat unexpected since the organism grows well in enrichment cultures without this material. The explanation of this apparent discrepancy may be that various associated microorganisms in enrichment cultures supply all of the essential growth factors.

Classification. Throughout this paper the caproic-acid-producing organism has been referred to as *C. kluyverii*. There can be no doubt that the organism belongs to the genus *Clostridium* as defined by Spray (1939) in view of its general morphological and physiological characteristics. It now remains to justify the creation of a new species.

The organism is distinguished morphologically by reason of its large size and negative gram-staining reaction. Its physiological

peculiarities are, however, more striking. It is unable to develop well in media suitable for many *Clostridium* species; milk, peptone, tryptone and glucose, for example, are inadequate substrates. Fairly good growth has been obtained only in media containing an extraordinarily high concentration of yeast autolysate and some alcohol. The further addition of peptone, tryptone or glucose has no effect. Diagnostically important also is the very small ammonia production. The characteristic metabolic reaction, one not previously observed with any other organism, is the conversion of ethyl alcohol to caproic acid. These properties seem to us to necessitate the separation of this bacterium from other described species of *Clostridium*. We therefore propose the specific name *Clostridium kluyverii* Barker and Taha *nov. spec.*, in honor of Prof. A. J. Kluyver in whose laboratory the organism was first discovered.

The characteristics of *C. kluyverii* may be summarized as follows: Straight to slightly bent rods, 0.9 to 1.1 microns by 3 to 11 microns, usually occurring singly, though sometimes in pairs or chains. Motile by means of peritrichous flagella. Spores oval, about 1.3 by 1.8 microns, terminal, causing swelling of the rods. Generally gram-negative, though sometimes weakly gram-positive. Strictly anaerobic. Growth slow, requiring about 2 to 3 days to become visible. Does not attack carbohydrates. Only very little ammonia is formed from organic nitrogenous compounds. Develops poorly or not at all in milk, tryptone, dilute yeast extract and similar materials. Moderate growth is obtained in pure culture in media containing a high concentration of yeast autolysate and ethyl alcohol. In association with *M. omelianskii*, it develops well in alcohol-containing media supplemented with dilute yeast autolysate. Characteristic metabolic reaction is the formation of caproic acid from ethyl alcohol and possibly other compounds. Hydrogen may be formed. Optimum temperature, about 34°C. Habitat: fresh water and marine muds.

SUMMARY

Nine strains of a new species of an anaerobic spore-forming bacterium, *Clostridium kluyverii*, have been isolated from fresh

water and marine muds. The organism is unique in forming caproic acid from ethyl alcohol, particularly when growing with the methane-producing bacterium, *Methanobacterium omelianskii*. Various morphological, physiological and nutritional characteristics are described.

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